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EFFECT OF CARBOHYDRATE ON AMINO ACID UTILIZATION OF CERTAIN BACTERIA

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That a utilizable carbohydrate influences the changes in protein and amino-acids in bacterial cultures, is so well known as to require no comment. The phenomenon has assumed undue prominence in the literature because various investigators do not agree in their interpretations. The two outstanding features of bacterial metabolism, which have been emphasized by Kendall, Walker and Day[1] in a series of experiments extending over several years, are: (1) Protein in the presence of a fermentable sugar is spared. (2) When sugar is present, protein metabolism is reduced to the minimum required for structural purposes. Herman and Rettger,[2] however, using a simple buffered medium in their series of experiments, came to the conclusion that "Kendall and Walker's conception that the presence of glucose delays the production of the proteolytic enzyme cannot be accepted. In the tests in which the buffer reagent was employed the proteolytic enzyme appeared as soon in the sugar media as in the plain bouillon." They ran parallel tests on mediums containing 0.2% and 0.4% dextrose, with 0.25% beef extract, 0.5% peptone, and 0.5% NaCl, one series without, and the other series with 0.5% K_2HPO_4. By following the changes by tests on successive days, an interesting sequence, when properly interpreted, is recorded in their table. Why they would limit the concentration of sugar to any percentage is not clear. The action of the buffer in their experiments is clearly to hold the concentration of hydrogen-ion below the toxic limit, and thus enable the organisms to exhaust the last trace of such small quantities of sugar. The sugar having been exhausted, the organisms of course then turn to the amino acids for their energy needs, which accounts for the sequence of changes shown in their table.

They conclude that "the presence of sufficient buffer in a medium encourages continued normal nitrogen metabolism." In other words, they argue for keeping the hydrogen-ion concentration nearer neutral-

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[1] Jour. Am. Chem. Soc., 1913, 135, p. 1201.

[2] J. Bacteriol., 3, 389, 1918.

ity. In compliance with this requirement, I have used 4% dextrose, with normal sodium hydroxid for titrating the acid as rapidly as formed with brom-cresol-purple in the culture as an indicator. After 12 hours the culture had to be watched continuously because of the rapid formation of acid, but after a period of 36 hours of this marked activity, the reaction became stationary. Although still maintained at the neutral point (P_H 7.5, by the hydrogen electrode) and kept for a period of 32 days, no evidence of "continued normal nitrogen metabolism" could be detected. The proteolytic enzyme had not appeared, indol was negative, ammonia formation was not greater than in the untitrated control, and sugar was still present.

Aside from the interesting fact that the activity of a culture can be arrested by the accumulation of products of its metabolism other than the toxic ion of hydrogen, perhaps in this instance the lactate ion principally, it is seen that even under the most favorable conditions of hydrogen-ion concentration, i. e., P_H 7.5, amino acid utilization for energy needs is definitely inhibited as long as sugar is available.

The paradoxic result of finding sugar in the phosphate medium but not in the 0.2% dextrose phosphate-free medium, requires explanation. Why was it not present in the unbuffered medium? That such a small trace of sugar was detected in the presence of 0.5% phosphate is most unusual. In regard to this, Berman and Rettger insist that "these results were indeed unexpected, and the tests were repeated, with identical results."

Whether sugar was present or absent, therefore, decides the interpretation of what follows. Here is the crux of the whole matter. The following points are offered as evidence against the claim that sugar was present: (1) It should be remembered that there is a common practice among bacteriologists of rendering beef infusion sugar free by incubating for 24 hours with dextrose fermenting organisms. How is such a practice to be justified if there is a "residuary carbohydrate" left in the culture even after 27 days' incubation? (2) To test the correctness of these findings one has only to kill such a culture, divide it into two portions, add at least 0.1% of sugar to one portion, and test both portions with Benedict's solution to discover that even with this extra 0.1% of sugar added to the "residuary carbohydrate" spoken of by these authors, the precipitate of cupric phosphate is so heavy in both tubes as to obscure entirely any positive sugar reaction. Even after repeated boiling and standing for 24 hours the tubes differ in no respect in appearance from an absolutely sugar free control. (3) When phosphate in such a concentration is known to be present, failure to remove it from the solution before testing for small traces of sugar, would be considered a serious oversight by any one acquainted with the limitations of Benedict's solution as a reagent for sugar in the presence of certain other substances.

Berman and Rettger claim that "fermentable sugars in moderate amounts do not affect the nitrogen metabolism of bacteria—under conditions of favorable environment," and that "the common belief in a so-called 'sparing action' of sugar in a protein medium is untenable in the light of these experiments. According to this idea protein is spared from all participation in the metabolism." I am unable to find anywhere in the reports of the work which they refute, any statement that "protein is spared from all participation in the metabolism," under any conditions. Growth requirements imply participation of protein in the metabolism.

The disagreement in our interpretations throughout probably originates from the experiment in which they found "a residuary carbohydrate" by Benedict's method, even in the presence of 0.5% phosphate, after 27 days' incubation, and though only 0.2% carbohydrate was present in the beginning. According to my experiments, the limiting hydrogen-ion concentration of B. proteus i. e., P_H 4.8, is sufficiently high to allow complete removal of 0.2% dextrose in 36 hours even in the usual (unbuffered) medium. Their table shows practically the same result in the 0.2% dextrose unbuffered medium, namely, positive sugar test up to 24 hours, but negative after 3 days. But why should it persist in the buffered medium? Phosphate should facilitate its removal rather than give rise to a "residuary carbohydrate."

Further disagreement arises from the fact that they do not make clear the distinction between protein hydrolysis and amino-acid utilization. These must be specified by more definite terms than the inclusive term "protein metabolism." Both should not be included when only one is meant. When the one occurs we see liquefaction without putrefaction; when the other occurs we find the formation of indol, H_2S and the genuine putrefactive changes characteristic of amino acid disintegration. They usually occur together but not necessarily. For example, many organisms which do not liquefy plain gelatin will produce a decided softening (acid hydrolysis) if a utilizable sugar is present. In other words, in the sugar-free gelatin cultures of, say, B. coli, putrefaction but not liquefaction occurs; in the sugar gelatin, liquefaction but not putrefaction, showing that either phase of "protein metabolism" may occur alone. To demonstrate this softening of gelatin by acid hydrolysis, one has only to acidify a tube of sterile gelatin medium to PH of about 4.5, with some acid, e.g., lactic, and compare its consistency after a few days at 37 C. with a similar control tube to which has been added a proportionate quantity of sterile water. Since the medium is kept sterile, this effect on the gelatin certainly is not one of "protein metabolism" and yet this softening of gelatin has often been erroneously offered as evidence that orotein metabolism occurs in the presence of sugar.

It is not impossible that certain organisms have been or will be found which could utilize both amino acids and sugars at the same time, but such strains will be the rarest exception to the rule, and their occurrence would not in any sense invalidate the general proposition set forth in the work of Kendall, Walker and Day as a valuable working hypothesis and basis of interpretation in metabolic studies.

SUMMARY

A culture of B. proteus containing sufficient carbohydrate shows no evidence of amino acid utilization, even though the reaction of the culture was maintained at neutrality during its entire period of active growth, and for an additional period of one month following cessation of activity.

The softening of gelatin occurring in sugar-gelatin medium is an acid rather than an enzymic-hydrolysis, and should not be interpreted as a part of protein metabolism.